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Thank you very much for consideration....

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Abstract

Valproic acid (VPA) is a commonly prescribed broad-spectrum antiepileptic agent and remains an important first-line therapeutic option for the management of various seizure disorders. Despite its established clinical efficacy, prolonged or high-dose exposure to VPA has been associated with adverse effects on the liver and kidneys. This study aimed to investigate the potential protective effects of myricetin (MYR) against VPA-induced tissue damage and to elucidate its possible underlying mechanisms. Rats were randomly assigned to four groups: control, VPA (500 mg/kg), VPA+MYR50 (500 mg/kg VPA + 50 mg/kg MYR), and VPA+MYR100 (500 mg/kg VPA + 100 mg/kg MYR). Treatments were administered for 15 days. PERK, IRE1, p38 MAPK, Bcl-2, JNK, TNF- α , and IL-1 β levels in liver and kidney tissues were determined by ELISA. Histopathological examinations were also performed. Additionally, molecular docking analyses were conducted to investigate the interactions of MYR with PERK and IRE1. VPA administration markedly increased hepatic and renal levels of PERK, IRE1, p38 MAPK, Bcl-2, MDA, TNF- α , and IL-1 β compared with the control group. MYR treatment attenuated these VPA-induced alterations, with both MYR doses showing protective effects on the examined parameters. Histopathological findings were consistent with the biochemical results, demonstrating an improvement in VPA-induced tissue damage following myricetin treatment. Molecular docking analysis further indicated favorable interactions of MYR with PERK and IRE1, supporting its potential modulatory effects on VPA-induced endoplasmic reticulum stress. MYR attenuated VPA-induced hepatic and renal injury, potentially through the modulation of ER stress, inflammatory, and apoptotic pathways. The findings indicate that MYR may have therapeutic potential in preventing or reducing VPA-associated tissue toxicity.

Keywords: Apoptosis, Endoplasmic Reticulum Stress, Inflammation, Molecular Docking, Myricetin, Valproic acid

Introduction

Valproic acid (VPA), a widely used antiepileptic drug worldwide, has a broad spectrum of therapeutic activity and is therefore used not only in the treatment of epilepsy but also in the management of mood disorders, bipolar disorder, and schizoaffective disorders, as well as in the treatment of neuropathic pain and migraine (Patel et al., 2022). Although VPA is generally well tolerated, numerous adverse effects have been reported, including potentially serious complications (Gziut & Thanacoody, 2025). Given that VPA often requires long-term use, understanding both its therapeutic benefits and potential adverse effects is of considerable importance for the effective monitoring and management of patients. Previous studies have demonstrated that VPA administration, particularly at high doses, may induce toxic damage to the liver and kidneys (Kadam et al., 2025; Bayrak et al., 2024).

Endoplasmic reticulum (ER) stress is primarily mediated through three major sensor proteins: protein kinase R-like endoplasmic reticulum kinase (PERK), inositol-requiring enzyme 1 (IRE1), and activating transcription factor 6 (ATF6) (Yan et al., 2018). ER stress is triggered by the accumulation of misfolded or unfolded proteins within the ER lumen and initiates an adaptive cellular response known as the unfolded protein response (UPR), which aims to restore cellular homeostasis. Initially, the UPR promotes adaptation by attenuating protein synthesis, enhancing protein refolding, and facilitating the removal of damaged or misfolded proteins. However, when ER stress persists for an extended period, sustained UPR activation may contribute to inflammation, apoptosis, and cellular injury (Reyes-Fermin et al., 2020).

PERK is a transmembrane protein involved in sensing ER stress and comprises a luminal stress-sensing domain and a cytoplasmic kinase domain. Under physiological conditions, PERK is maintained in an inactive state through its interaction with binding immunoglobulin protein (BiP) (Wiseman et al., 2022). Upon the development of ER stress and the accumulation of misfolded proteins, BiP preferentially binds to these proteins and dissociates from PERK. This dissociation promotes PERK dimerization and subsequent autophosphorylation, thereby activating PERK-mediated signaling. Activated PERK phosphorylates the alpha subunit of eukaryotic initiation factor 2 (eIF2 α). Phosphorylation of eIF2 α suppresses global protein synthesis, thereby reducing the influx of newly synthesized proteins into the ER, while simultaneously facilitating the selective translation of specific stress-responsive proteins. Thus,

the PERK–eIF2 α axis plays a critical role in cellular adaptation to ER stress and the maintenance of protein homeostasis (Read & Schröder, 2021; Hetz et al., 2020).

IRE1 is one of the earliest sensors activated in response to ER stress. IRE1 possesses two main enzymatic activities: kinase activity and endoribonuclease (RNase) activity (Durose et al., 2006). IRE1 contributes to an adaptive response aimed at alleviating ER stress by enhancing the protein-folding capacity of the ER, while also contributing to the initiation of mitochondria-mediated apoptotic signaling. Thus, IRE1 acts as a bidirectional regulatory mechanism that attempts to promote cell survival under moderate stress but can trigger inflammatory responses and apoptosis under conditions of excessive or prolonged stress (Sano & Reed, 2013).

The literature indicates that, in addition to its essential role in protein metabolism, the ER functions as a dynamic regulator at the center of cellular stress responses and inflammatory signaling networks (Grootjans et al., 2016). ER stress has been shown to contribute substantially to inflammatory processes, including the activation of immune responses and the production of proinflammatory cytokines. The accumulation of proinflammatory cytokines, such as interleukin-1 beta (IL-1 β) and tumor necrosis factor-alpha (TNF- α), has been associated with the activation of the UPR. Moreover, a bidirectional feedback loop has been proposed, in which inflammation exacerbates ER stress, while ER stress, in turn, amplifies inflammatory signaling (Duvigneau et al., 2019).

Throughout their lifespan, cells are exposed to a wide range of stressors under both physiological and pathological conditions. Effective regulation of these stress responses is essential for maintaining cellular and organismal survival. The ability of cells to appropriately and coordinately adapt to diverse stress conditions depends on the activation and integration of multiple intracellular signaling networks (Martínez-Limón et al., 2020). The p38 mitogen-activated protein kinase (p38 MAPK) signaling pathway is activated in response to a broad spectrum of cellular stressors, including heat shock, osmotic stress, oxidative stress, proinflammatory cytokines, DNA-damaging agents, ultraviolet (UV) radiation, γ -radiation, hypoxia, and ischemia (Canovas & Nebreda 2021).

c-Jun N-terminal kinase (JNK), a member of the mitogen-activated protein kinase (MAPK) family, is an important mediator of apoptosis during the later stages of the ER stress response (Weiss et al., 2000). Sustained ER stress can activate JNK through the IRE1-dependent signaling pathway, whereas JNK may also be stimulated by extracellular stress signals

transmitted through cell-surface receptors. Although these pathways are initiated by distinct stimuli, both can converge on JNK activation and promote downstream pro-apoptotic signaling. Activated JNK can phosphorylate B-cell lymphoma 2 (Bcl-2), thereby attenuating its anti-apoptotic activity and shifting the cellular balance toward apoptosis (Sano & Reed, 2013). Thus, persistent activation of the IRE1–JNK axis may represent an important molecular link between prolonged ER stress and the induction of apoptosis.

As an anti-apoptotic protein, Bcl-2 preserves mitochondrial membrane integrity and prevents the release of cytochrome c into the cytoplasm, thereby suppressing caspase activation and contributing to the protection of cells against apoptosis. The balance between Bcl-2 and pro-apoptotic proteins is critical in determining whether cells undergo survival or apoptotic processes. Therefore, a reduction in Bcl-2 levels may be associated with the activation of the mitochondrial apoptotic pathway and increased cellular damage (Vogler et al., 2025).

Free radicals can readily interact with unsaturated fatty acids and cholesterol in cell membranes, leading to lipid peroxidation. Malondialdehyde (MDA), one of the major end products and widely used indicators of lipid peroxidation, can interact with intracellular proteins, lipids, and nucleic acids. Elevated MDA levels are considered an important indicator of increased lipid peroxidation and the resulting oxidative damage (Morales & Munné-Bosch, 2019).

Myricetin (MYR) is a flavonol widely distributed in nature, predominantly occurring in the form of glycosides rather than as free aglycones, and is commonly found in fruits and vegetables. Structurally, MYR consists of two aromatic rings, designated as A and B, which are linked by a three-carbon chain that forms a cyclic C ring. The high number of hydroxyl groups in its chemical structure has been suggested to contribute substantially to its potent antioxidant activity (Agraharam et al., 2022). Owing to its strong antioxidant properties, MYR has been reported to act as a potential modulator of various cellular processes and may play an active role in pathways involved in cell survival, proliferation, differentiation, migration, homeostasis, and apoptosis (Javed et al., 2022).

The present study aimed to investigate the protective effects of MYR against VPA-induced liver and kidney injury, with particular emphasis on its effects on ER stress, inflammation, and apoptosis. The extent of hepatic and renal tissue injury and the potential protective effects of MYR were also evaluated histopathologically to provide morphological evidence supporting

the biochemical and molecular findings. In addition, the binding mechanisms of synthetic inhibitors targeting the kinase domain of the PERK enzyme (GSK2606414, GSK2656157, and AMG44) and the naturally occurring flavonoid MYR were analyzed using a molecular docking approach, with ATP serving as a reference ligand.

Methods

Animals and experimental design

Atatürk University Local Ethics Committee for Animal Experiments approved this experimental protocol (2025/51). A total of 36 male Wistar–Albino rats, weighing 250–300 g, were maintained under standard laboratory conditions (21–22°C, 60 ± 5% relative humidity, and a 12 h light/12 h dark cycle) with ad libitum access to standard laboratory chow and water. The animals were randomly divided into four experimental groups, each consisting of nine rats (n = 9).

The rats were randomly divided into four groups: 1 ml of saline via oral gavage (Control group), 500 mg/kg VPA (dissolved in distilled water) by intraperitoneally (VPA group) (Terim Kapakin et al., 2025), 500 mg/kg VPA plus 50 mg/kg MYR via oral gavage (VPA+MYR50 group), or 500 mg/kg VPA plus 100 mg/kg MYR (VPA+MYR100 group) (Wang et al., 2023; Yang et al., 2024). The treatments were applied for 15 days. All rats in the experimental groups were sacrificed 24 hours after the final treatment. The VPA (CAS No: 99-66-1) and MYR (Cas No: 529-44-2, saflık %97) were purchased from Sigma Aldrich (Sigma Chemical Co., St. Louis, MO, USA).

At the end of the experimental period, blood, liver, and kidney samples were collected for biochemical and histopathological analyses. Prior to intracardiac blood collection, the rats were anesthetized with ketamine (80 mg/kg; Ketalar®, 50 mg/mL, Eczacıbaşı, Istanbul, Türkiye) and xylazine (10 mg/kg; Rompun®, 2%, Bayer, Istanbul, Türkiye). Following blood collection, all animals were euthanized by exsanguination under deep anesthesia. The abdominal cavity was then opened, and the liver and kidney tissues were carefully excised for subsequent analyses.

Biochemical analysis

Blood samples were centrifuged at 4,000 × g for 15 min, and the obtained serum was used for biochemical analyses. Serum glucose, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine,

total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) levels were determined.

Determination of tissue malondialdehyde levels

Liver and kidney malondialdehyde (MDA) levels were determined according to the method described by Ohkawa et al. (1979). This assay is based on the spectrophotometric measurement of the pink-red chromogen formed by the reaction of MDA, a major end product of polyunsaturated fatty acid (PUFA) peroxidation, with thiobarbituric acid (TBA) under high-temperature acidic conditions. The absorbance of the resulting MDA–TBA complex was measured at 532 nm. Protein concentrations were determined using the Bradford method (1976), with bovine serum albumin (BSA) as the standard. MDA levels were normalized to protein content and expressed as nmol/mg protein.

ELISA analysis of tissue biomarkers

The concentrations of PERK, IRE1, p38 MAPK, Bcl-2, JNK, TNF- α , and IL-1 β in liver and kidney tissue homogenates were quantified using commercially available enzyme-linked immunosorbent assay (ELISA) kits (BT Lab, Shanghai, China) according to the manufacturer's instructions. The assay is based on the specific binding of antigens to their corresponding antibodies, with an enzyme-conjugated detection system used to generate a measurable signal. Following the addition of the substrate, enzyme activity was quantified spectrophotometrically according to the manufacturer's protocol. The IRE1 and TNF- α results were expressed as ng/L, whereas the PERK, p38 MAPK, Bcl-2, JNK, and IL-1 β result was expressed as ng/mL. The catalog numbers of the ELISA kits were as follows: PERK (Cat. No. E1526Ra), IRE1 (Cat. No. E2893Ra), p38 MAPK (Cat. No. E2473Ra), Bcl-2 (Cat. No. E0037Ra), JNK (Cat. No. E0048Ra), TNF- α (Cat. No. E0764Ra), and IL-1 β (Cat. No. E0119Ra).

Histopathologic examination

The liver and kidney tissue samples were fixed in 10% buffered formalin and routinely processed for histopathological examination by embedding in paraffin wax. Tissue sections were cut 4 μ m in thickness and stained by the Hematoxylin–Eosin (HXE) for observation under a light microscope (Gundogdu et al., 2023).

Tissue sections were evaluated by high-power light microscopic examination using an Olympus Bx51 with a DP72 camera system. All tissue sections were evaluated blindly by two

independent observers. Each specimen was examined in 10 randomly selected areas of approximately an X40 objective. The scores were derived semi-quantitatively on the preparations from each rat. Lesion severity was graded based on the percentage of affected area as follows Grade 0 (–): no lesion, 0%; Grade 1 (+1): <25%; Grade 2 (+2): 25–50%; Grade 3 (+3): >50–75%; Grade 4 (+4): >75% involvement (Terim Kapakin et al., 2024).

Protein and ligand preparation for PERK

The crystal structure of PERK (PDB ID: 4G31) was selected as the structural model for the molecular docking analyses and prepared using PyMOL. Protein preparation was performed based on the *Homo sapiens* reference sequence (NP_004827.4). To ensure accurate calculation of molecular interactions, appropriate atomic charge assignments were applied during the preparation process. Kollman charges were assigned to the target protein to represent the ionization states of the amino acid residues, whereas Gasteiger charges were assigned to the test ligands to define their partial atomic charge distributions. Subsequently, all molecular structures were converted into the PDBQT format using MGLTools, and molecular flexibility was preserved by defining the detect root and torsion parameters.

Docking parameters for PERK

The molecular docking simulations were carried out using AutoDock 4.2, employing the Lamarckian Genetic Algorithm (LGA) with the default parameter set. The molecular docking grid and algorithm parameters are presented in Table 1.

Preparation of IRE1 complexes

The active conformation (DICI form) of the human IRE1 α kinase domain was prepared using the crystal structure with PDB ID 6W3B obtained from the Protein Data Bank (PDB). Missing regions in the crystal structure were reconstructed by homology modeling using the SWISS-MODEL server, and the residue numbering was normalized to correspond to residues 562–832 of the UniProt reference sequence (O75460). The prepared receptor structure was subsequently used for molecular docking, in which (i) ATP, in the presence of two Mg²⁺ ions, was employed as the positive control, and (ii) myricetin was used as the test ligand. Both ligands were docked into the nucleotide-binding pocket of the protein. Force field parameters for the ligands were generated using the Antechamber module of AmberTools. Atom types for both ligands were

assigned according to the General Amber Force Field 2 (GAFF2), and partial atomic charges were calculated using the AM1-BCC (Austin Model 1 with Bond Charge Correction) method.

ATP was parameterized with a net charge of -4 , consistent with physiological pH conditions, whereas myricetin was treated as a neutral molecule (net charge = 0). Missing force field parameters were generated, and the corresponding `frmod` files were created using the `parmchk2` utility. The topology and coordinate files of the complexes were generated using the `tleap` module implemented in the Amber20 software package. The `ff14SB` force field was applied to the protein, GAFF2 to the ligands, and the TIP3P water model was used to represent solvent molecules. To accurately describe the behavior of Mg^{2+} ions in the aqueous environment, the `ions1lm_126_tip3p` and `ionsjc_tip3p` ion parameter sets were incorporated into the system.

Each system was solvated in a truncated octahedral box of TIP3P water molecules, ensuring a minimum distance of 8.0 Å between the solute and the box boundaries. The systems were electrically neutralized by adding an appropriate number of Na^+ and Cl^- counterions.

Molecular dynamics simulation of IRE1

Energy minimization

Following the generation of the topology and initial coordinate files for the protein–ligand complexes, energy minimization was performed using the `pmemd` module implemented in the Amber20 software package to eliminate unfavorable atomic contacts and reduce the potential energy of the systems to a local minimum. The minimization protocol consisted of a total of 100,000 cycles (`maxcyc` = 100,000) employing a hybrid combination of the Steepest Descent and Conjugate Gradient algorithms. During the initial 10,000 cycles (`ncyc` = 10,000), the Steepest Descent algorithm was applied to efficiently remove severe steric clashes and high-energy contacts. This was followed by 90,000 cycles of the Conjugate Gradient algorithm (`ntmin` = 1) to achieve convergence toward a more stable energy minimum.

Non-bonded interactions, including electrostatic and van der Waals forces, were calculated using a 9.0 Å cutoff distance (`cut` = 9.0). All simulations were carried out under periodic boundary conditions (PBC) using the constant-volume (NVT) approach (`ntb` = 1). During the minimization stage, all degrees of freedom were allowed to relax without positional restraints.

No bond constraints were applied ($\text{ntc} = 1$), and all force components were explicitly evaluated ($\text{ntf} = 1$).

Heating

Following energy minimization, the protein–ligand complexes were gradually heated from 0 K to the target temperature of 300 K using the `pmemd` module under the constant-volume, constant-temperature (NVT) ensemble. The heating phase was carried out over 1 ns, during which the temperature was increased linearly from 0 K to 300 K over 1,000,000 simulation steps using the `nmropt = 1` option together with predefined weighting functions (&wt).

Temperature regulation was achieved using the Langevin thermostat ($\text{ntt} = 3$) with a collision frequency of 10.0 ps^{-1} ($\text{gamma_ln} = 10.0$), ensuring uniform distribution of kinetic energy throughout the system while minimizing thermal fluctuations. Throughout the heating stage, all bonds involving hydrogen atoms were constrained using the SHAKE algorithm ($\text{ntc} = 2$, $\text{ntf} = 2$) to improve numerical stability and permit a larger integration time step. As in the minimization stage, non-bonded interactions were evaluated using a $9.0 \text{ \AA}$ cutoff distance ($\text{cut} = 9.0$), and periodic boundary conditions ($\text{ntb} = 1$) were maintained throughout the simulation.

Production Molecular Dynamics Simulations

Following the heating and equilibration stages, 500 ns production molecular dynamics (MD) simulations were performed to investigate the structural dynamics and binding stability of both protein–ligand complexes. All production simulations were carried out using the GPU-accelerated `pmemd.cuda` module implemented in the Amber20 software package under the isothermal–isobaric (NPT) ensemble.

For each complex, the simulations were performed with a 2 fs integration time step ($\text{dt} = 0.002 \text{ ps}$) for a total simulation time of 500 ns (250,000,000 simulation steps). To ensure simulation continuity and computational efficiency, the production run was divided into 100 ns consecutive segments using the restart option ($\text{irest} = 1$, $\text{ntx} = 5$). The simulation parameters were optimized as described below.

Temperature Control

The system temperature was maintained at 300 K using the Langevin thermostat ($\text{ntt} = 3$) with a collision frequency of 2.0 ps^{-1} ($\text{gamma_ln} = 2.0$).

Pressure Control

System pressure was maintained at 1 bar using the Monte Carlo barostat ($\text{barostat} = 2$), which provides efficient pressure regulation in GPU-accelerated simulations.

Non-bonded Interaction Calculations

A $9.0 \text{ \AA}$ cutoff distance was applied for short-range non-bonded interactions, whereas long-range electrostatic interactions were treated using the Particle Mesh Ewald (PME) method.

Constraints and Trajectory Analysis

All bonds involving hydrogen atoms were constrained using the SHAKE algorithm to maintain numerical stability throughout the simulations. Trajectory coordinates were recorded every 100 ps ($\text{ntwx} = 50,000$), while potential energy values were saved every 10 ps. All trajectory and energy output files were stored in NetCDF format to optimize storage efficiency.

The trajectories generated during the 500 ns simulations were analyzed using the `cpptraj` module of the AmberTools20 package. To evaluate the structural stability and equilibration of the systems throughout the simulations, root mean square deviation (RMSD) analysis was performed. RMSD values were calculated using the $\text{C}\alpha$ atoms of the protein, with the energy-minimized initial structure serving as the reference.

The local flexibility and dynamic fluctuations of individual amino acid residues were assessed by root mean square fluctuation (RMSF) analysis. RMSF values were calculated based on the $\text{C}\alpha$ atoms, with particular emphasis on evaluating the influence of myricetin binding on the dynamics of the αC -helix and the activation loop (A-loop; residues 711–725).

The Lys599–Glu612 salt bridge, a key structural feature defining the active (DICI) conformation of the IRE1 α kinase domain, was monitored throughout the simulations. To assess the stability of this interaction, the distance between the terminal NZ atom of the Lys599 side chain and the CD atom of the Glu612 carboxylate group was calculated as a function of simulation time. Differences in this distance between the ATP-bound and myricetin-bound

complexes were used as the primary structural criterion to determine whether myricetin induced an outward displacement of the α C-helix toward the inactive (DICO) conformation or otherwise disrupted the active kinase conformation.

Statistical analysis

Differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test for multiple comparisons. A *p* value of < 0.05 was considered statistically significant. Data are presented as mean $\pm$ standard deviation (SD).

Results

Compared with the control group, VPA administration resulted in significant increases in the plasma concentrations of glucose, TC, TG, LDL, AST, ALT, ALP and BUN ($p < 0.05$). In contrast, no statistically significant difference was observed in plasma creatinine levels between the control and VPA-treated groups ($p > 0.05$). Treatment with MYR at both 50 mg/kg (MYR50) and 100 mg/kg (MYR100) significantly reduced the elevated levels of TC, TG, LDL, AST, ALT, ALP and BUN compared with the VPA group ($p < 0.05$). The VPA+MYR50 group exhibited a statistically significant reduction in plasma glucose levels compared with the VPA group ($p < 0.05$). However, plasma glucose levels were also lower in the VPA+MYR100 group than in the VPA group, this reduction did not reach statistical significance ($p > 0.05$). The results are shown in Table 2.

Hepatic TNF- α and IL-1 β levels in VPA group were significantly increased compared to control group ($p < 0.05$), indicating a pronounced inflammatory response in the liver. Treatment with MYR50 and MYR100 significantly attenuated the VPA-induced increases in TNF- α and IL-1 β levels compared with the VPA group ($p < 0.05$) (Fig. 1). MYR50 produced a greater reduction in both inflammatory markers than MYR100, with TNF- α levels in the VPA+MYR50 group approaching those observed in the control group.

Hepatic PERK levels in the VPA group were significantly increased compared with the control group ($p < 0.05$). The VPA + MYR50 and VPA + MYR100 groups had significantly lower hepatic PERK levels than the VPA group ($p < 0.05$). Similarly, hepatic IRE1 levels were significantly higher in the VPA group compared with the control group ($p < 0.05$), whereas both

the VPA + MYR50 and VPA + MYR100 groups exhibited significantly lower IRE1 levels than the VPA group ($p<0.05$) (Fig. 2).

Hepatic JNK levels were higher in the VPA group than in the control group; however, the difference was not statistically significant ($p>0.05$). No significant differences were observed in JNK levels between the VPA group and the VPA + MYR50 or VPA + MYR100 groups ($p>0.05$). Hepatic p38 MAPK levels were significantly elevated in the VPA group compared with the control group ($p<0.05$), while treatment with MYR50 and MYR100 significantly reduced p38 MAPK levels compared with the VPA group ($p<0.05$) (Fig. 3).

Hepatic Bcl-2 levels were significantly increased in the VPA group compared with the control group ($p<0.05$). Although Bcl-2 levels were lower in the VPA+MYR50 and VPA+MYR100 groups than in the VPA group, these differences were not statistically significant ($p>0.05$). In contrast, hepatic MDA levels were significantly elevated in the VPA group compared with the control group ($p<0.05$), whereas treatment with MYR50 and MYR100 significantly reduced MDA levels compared with the VPA group ($p<0.05$) (Fig. 4).

Renal TNF- α and IL-1 β levels in VPA group were significantly increased compared to control group ($p<0.05$). Treatment with MYR50 and MYR100 significantly attenuated the VPA-induced increases in TNF- α and IL-1 β levels compared with the VPA group ($p<0.05$) (Fig. 5). MYR50 produced a greater reduction in both inflammatory markers than MYR100, with TNF- α levels in the VPA+MYR50 group approaching those observed in the control group.

Renal PERK levels were significantly increased in the VPA group compared with the control group ($p<0.05$). Treatment with MYR50 and MYR100 significantly reduced PERK levels compared with the VPA group ($p<0.05$). Similarly, renal IRE1 levels were significantly higher in the VPA group compared with the control group ($p<0.05$), whereas both the VPA + MYR50 and VPA + MYR100 groups exhibited significantly lower IRE1 levels than the VPA group ($p<0.05$) (Fig. 6). Both MYR50 and MYR100 exerted comparable effects on renal PERK and IRE1 levels.

Renal JNK levels did not differ significantly among the experimental groups ($p>0.05$). In contrast, VPA administration significantly increased renal p38 MAPK levels compared with the control group ($p<0.05$). Treatment with both MYR50 and MYR100 significantly attenuated the VPA-induced elevation in renal p38 MAPK levels ($p<0.05$). Both MYR50 and MYR100

exhibited comparable effects on renal p38 MAPK levels, whereas neither treatment significantly altered renal JNK levels (Fig. 7).

VPA administration significantly increased renal Bcl-2 levels compared with the control group ($p<0.05$). The VPA+MYR50 and VPA+MYR100 groups showed significantly lower renal Bcl-2 levels compared with the VPA group ($p<0.05$). Similarly, renal MDA levels were significantly increased in the VPA group compared with the control group ($p<0.05$), whereas MDA levels were significantly decreased in both the VPA+MYR50 and VPA+MYR100 groups compared with the VPA group ($p<0.05$). The VPA+MYR50 and VPA+MYR100 groups exhibited comparable effects on renal Bcl-2 and MDA levels (Fig. 8).

Histopathological examination of the liver tissue from the control group revealed no detectable pathological alterations. In contrast, the VPA-treated group exhibited marked degenerative and necrotic changes in hepatocytes. In addition, mononuclear inflammatory cell infiltration, predominantly composed of lymphocytes, was observed, particularly in the periportal and perivascular regions, accompanied by pronounced Kupffer cell activation. In the myricetin-treated groups (VPA + MYR50 and VPA + MYR100), these histopathological alterations were markedly attenuated compared with the VPA group. Specifically, hepatocellular degeneration and necrosis were substantially reduced, inflammatory cell infiltration was significantly diminished, and Kupffer cell activation was markedly alleviated (Fig. 9).

Compared with the control group, VPA administration resulted in a marked and statistically significant increase in the histopathological scores of degeneration, inflammation, and necrosis in liver tissue ($p<0.001$) (Fig. 10). In contrast, treatment with MYR (MYR50 and MYR100) significantly attenuated these histopathological alterations. These findings suggest that myricetin exerts a protective effect against VPA-induced liver injury. Moreover, the lower severity of histopathological lesions observed in the MYR100 group compared with the MYR50 group indicates that the hepatoprotective effect of MYR may be dose-dependent. The absence of a significant difference between the control group and the MYR-treated groups (ns) further suggests that myricetin alone does not induce any adverse histopathological alterations in liver tissue.

Histopathological examination of the kidney tissue revealed normal renal architecture in the control group, with no detectable pathological alterations (Fig. 11). In contrast, the VPA-treated group exhibited prominent degenerative changes characterized by marked vacuolization and hydropic degeneration of the proximal tubular epithelial cells, accompanied by tubular necrosis. In addition, tubular dilatation was observed in several renal tubules, and the tubular lumina were filled with degenerated and desquamated epithelial cells. Glomerular alterations included glomerular atrophy in some glomeruli and thickening of the glomerular basement membrane in others. Furthermore, mononuclear inflammatory cell infiltration, predominantly composed of lymphocytes, was evident within the renal interstitium.

In the MYR-treated groups (VPA+MYR50 and VPA+MYR100), these histopathological alterations were partially reversed compared with the VPA group. Degenerative changes and necrosis in the tubular epithelial cells were markedly reduced, accompanied by a noticeable decrease in interstitial inflammatory cell infiltration. Likewise, glomerular basement membrane thickening and glomerular atrophy were less pronounced in the myricetin-treated groups. Notably, the improvement observed in the MYR100 group was more prominent than that in the MYR50 group, suggesting a dose-dependent protective effect of MYR. Quantitative histopathological scoring demonstrated that VPA administration induced significant increases in the scores of degeneration, inflammation, and necrosis compared with the control group ($p < 0.001$) (Fig. 12). These findings indicate that MYR effectively attenuated VPA-induced renal histopathological damage, with the higher dose (100 mg/kg) providing greater histological protection than the lower dose (50 mg/kg).

Binding affinity analysis of PERK

The binding free energies obtained from the molecular docking simulations indicated that the tested compounds exhibited higher binding affinities than the endogenous ligand, ATP. Analysis of the calculated binding free energy (ΔG) values demonstrated that myricetin possesses a remarkable binding affinity toward the PERK kinase domain. The binding free energy of myricetin was calculated to be -7.52 kcal/mol, which was slightly more favorable than that of ATP (-7.20 kcal/mol). This finding thermodynamically supports the potential of MYR to act as an ATP-competitive inhibitor of PERK by preferentially occupying the ATP-binding pocket (Table 3).

Functional region interactions of PERK

The binding poses of the ligands were evaluated with respect to the conserved structural motifs that are essential for the kinase activity of PERK (Table 4). AMG44 exhibited the most favorable binding affinity among all tested ligands, with a binding free energy of -14.47 kcal/mol, forming the most stable protein–ligand complex. The GSK derivatives (-12.77 and -12.36 kcal/mol) demonstrated binding orientations fully consistent with the reference crystal structures 4G31, 4M7I, and 4X7N, thereby validating the reliability of the docking protocol. The binding free energy of myricetin (-7.52 kcal/mol) was slightly more favorable than that of ATP (-7.20 kcal/mol), supporting its potential to function as an ATP-competitive inhibitor of PERK. Structural analysis revealed that the planar scaffold of myricetin adopts a binding orientation similar to that of the adenine ring of ATP within the ATP-binding pocket. However, unlike AMG44 and the GSK derivatives, MYR lacks an extended molecular scaffold capable of penetrating into the back pocket of the PERK kinase domain. This structural limitation likely accounts for its comparatively lower binding affinity relative to the synthetic inhibitors.

Molecular docking analysis confirmed that AMG44 and the GSK-series inhibitors possess a high binding affinity for the PERK kinase domain, consistent with their potent inhibitory activity. As a naturally occurring flavonoid, myricetin exhibited a binding orientation similar to that of ATP within the ATP-binding pocket, suggesting its potential to inhibit PERK through an ATP-competitive mechanism. However, unlike the synthetic inhibitors, myricetin does not achieve the deep-pocket stabilization required for stronger binding interactions. Structural analysis further revealed that the planar polyphenolic scaffold of myricetin occupies a region analogous to the adenine moiety of ATP within the ATP-binding site (Fig. 13).

Structural stability of IRE1: RMSD comparison

Analysis of the molecular dynamics simulation results demonstrated that both complexes maintained their overall structural integrity throughout the 500 ns simulation period. However, the myricetin-bound complex exhibited lower and more stable backbone RMSD values (approximately 2.0 – 2.5 Å) compared with the ATP-bound complex (approximately 2.5 – 2.8 Å), indicating enhanced structural stability upon myricetin binding (Fig. 14).

The most pronounced difference was observed in ligand stability. While the RMSD values of ATP fluctuated around 2.2 Å throughout the simulation, MYR remained highly stable,

maintaining an RMSD of approximately 1.8 Å. These findings indicate that MYR adopts a more geometrically favorable and stable binding pose within the ATP-binding pocket than the native ligand ATP, thereby providing tighter stabilization of the binding site. This enhanced conformational stability is consistent with the potential of MYR to act as an effective ATP-competitive inhibitor of the IRE1 α kinase domain.

Regional flexibility and domain dynamics of IRE1: B-factor analysis

The B-factor profiles demonstrated that, in both complexes, the RNase domain (residues 833–962) exhibited greater flexibility than the kinase domain, consistent with its intrinsically dynamic nature. Notably, in the presence of MYR, critical structural elements within the kinase domain, particularly the α C-helix and the activation loop (A-loop), displayed reduced flexibility compared with the ATP-bound complex. Whereas ATP preserves the conformational mobility required for catalytic function, MYR stabilizes these regulatory regions, thereby restricting the conformational transitions necessary for kinase activation. These findings suggest that MYR suppresses the structural dynamics of the IRE1 α kinase domain, providing a mechanistic basis for its potential inhibitory effect (Fig. 15).

Structure–Function Analysis of IRE1 α

Comparison of the critical interaction distances revealed that myricetin exhibits the characteristics of a Type I kinase inhibitor and provides mechanistic insight into its inhibitory action on IRE1 α . Throughout the simulation, the Lys599–Glu612 salt bridge remained below the 4.0 Å threshold in both the ATP-bound and myricetin-bound complexes, indicating preservation of the active (DICI) conformation (Fig. 16). These findings suggest that, rather than disrupting the overall protein architecture, myricetin occupies the ATP-binding pocket in the active kinase conformation, thereby exerting its inhibitory effect through a competitive inhibition mechanism by competing with ATP for binding to the catalytic site.

Catalytic Locking (Lys599–Asp711): In the ATP-bound complex, the Lys599–Asp711 distance fluctuated between approximately 3.5 and 8.0 Å, reflecting the conformational flexibility required for catalytic activity. In contrast, in the myricetin-bound complex, this distance remained remarkably stable at approximately 3.7 Å throughout the simulation. These findings suggest that myricetin effectively locks the catalytic lysine (Lys599) and the DFG

aspartate (Asp711) into a fixed conformation, thereby mechanically restricting the conformational dynamics required for efficient phosphoryl transfer and kinase catalysis.

Discussion

Adverse drug effects have emerged as an important health concern, particularly in terms of achieving successful therapeutic outcomes in disease management. VPA, which is widely used in the treatment of epilepsy and other neurological disorders, is frequently associated with hepatic and renal injury (Kadam et al., 2025; Terim-Kapakin et al., 2025). This represents a significant risk to the safe use of the drug. Therefore, a major goal during treatment is to minimize the adverse effects of VPA while maintaining safe and effective therapy. In this context, natural compounds have long been investigated for their potential to mitigate drug-induced adverse effects. In the present study, we investigated the biochemical mechanisms underlying the detrimental effects of VPA on the liver and kidneys and evaluated the effects of MYR, a potent antioxidant compound, on these mechanisms.

Biochemical parameters are important indicators for evaluating liver and kidney function, as well as identifying tissue damage and metabolic alterations. Changes in these parameters can provide valuable information regarding hepatic and renal functional alterations that may occur following drug exposure. In the present study, VPA administration resulted in statistically significant increases in AST, ALT, ALP, BUN, TG, TC, and LDL levels compared with the control group. Treatment with both MYR50 and MYR100 attenuated these VPA-induced elevations, indicating a potential protective effect of MYR against VPA-associated hepatic, renal, and metabolic alterations. MYR50 exerted a more pronounced protective effect than MYR100, as evidenced by its greater ability to reduce the elevated levels of these biochemical parameters. These findings suggest that MYR, particularly at the 50 mg/kg dose, may effectively mitigate VPA-induced biochemical disturbances associated with liver and kidney injury.

The endoplasmic reticulum (ER) is a key cellular organelle that plays a central role in protein synthesis, folding, and maturation. The accumulation of misfolded or unfolded proteins within the ER lumen disrupts ER homeostasis and leads to the development of ER stress. PERK and IRE1 are important sensors involved in the cellular response to ER stress (Kopp et al., 2019). PERK is a key UPR sensor that becomes activated in response to ER stress and aims to

reduce cellular burden by transiently suppressing protein synthesis. However, prolonged activation of the PERK pathway may contribute to the initiation of apoptotic processes. Therefore, PERK activation is considered a critical step in determining the balance between the adaptive cellular response and cell death (Ron D, Walter, 2007; Hetz & Papa, 2018).

IRE1 is a dual-function ER stress sensor that is activated in response to ER stress and can regulate both the adaptive UPR response and, depending on the severity of the stress, apoptotic signaling. While transient activation of IRE1 supports cell survival, prolonged or excessive activation has been associated with inflammation and apoptosis (Wang & Kaufman, 2014). In the literature, increased levels of PERK and IRE1 have been reported in VPA-induced toxicity models, and these elevations have been suggested to reflect the cellular response associated with ER stress. Kandemir et al. (2022) reported that VPA administration exacerbated endoplasmic reticulum (ER) stress by increasing PERK and IRE1 levels. Similarly, in another study reported in the literature, Palsamy et al. (2014) demonstrated that VPA induces ER stress and activates the unfolded protein response through the activation of key ER stress sensors, including PERK and IRE1 α .

In the present study, evaluation of VPA-induced tissue injury revealed a marked increase in PERK and IRE1 levels in both the liver and kidney tissues. Considering that PERK and IRE1 are key sensors of endoplasmic reticulum (ER) stress, these findings suggest that VPA exposure markedly activates ER stress in both tissues. Moreover, the significant reduction in PERK and IRE1 levels following MYR treatment indicates that MYR may exert a potent protective effect against VPA-induced ER stress. When the effects of the different doses were compared, both MYR50 and MYR100 produced marked and consistent effects on PERK and IRE1 levels, suggesting that both doses of MYR effectively attenuated ER stress.

ER stress and inflammation are closely interconnected cellular processes that contribute to the progression of tissue injury. Persistent ER stress activates the UPR, particularly the PERK and IRE1 signaling pathways, which initially function as adaptive mechanisms to restore cellular homeostasis. However, prolonged or excessive activation of these pathways can stimulate pro-inflammatory signaling cascades, thereby promoting the expression of inflammatory mediators such as TNF- α and IL-1 β (Zhang & Kaufman, 2008; Grootjans ET AL., 2016).

Inflammatory cytokines can further disrupt ER function and aggravate disturbances in protein folding, thereby establishing a self-reinforcing interaction between ER stress and inflammation. Consequently, persistent ER stress may not only promote cellular dysfunction and apoptosis but may also contribute substantially to the development and progression of inflammation-associated tissue injury (Mei et al., 2013). The mechanisms underlying VPA-induced hepatotoxicity and nephrotoxicity are complex and multifactorial. VPA-induced toxicity is thought to be associated with the activation of inflammatory responses, which may substantially contribute to tissue injury (Mohammed et al., 2021).

Cytokines are biologically active signaling molecules primarily produced by immune cells that regulate immune responses and communicate with various cellular and physiological systems to preserve homeostasis. These proteins play essential roles in numerous cellular processes, including inflammation, intercellular signaling, cell differentiation and development, and cellular survival (Kothur et al., 2016). IL-1 β and TNF- α are key mediators involved in the pathogenesis of various inflammatory diseases. Given that IL-1 β is commonly activated under conditions in which TNF- α is produced, it has been proposed that IL-1 β may modulate TNF- α -induced proinflammatory responses in epithelial cells by influencing TNF receptor shedding and its expression on the cell surface (Saperstein et al., 2009).

Oztopuz et al. (2020) reported that VPA administration induced hepatic injury in rats and was accompanied by significant increases in TNF- α and IL-1 β levels. Similarly, Alhusaini et al. (2024) demonstrated that VPA-induced renal injury was associated with marked elevations in TNF- α and IL-1 β levels. Collectively, these findings suggest that VPA-induced tissue injury may be closely associated with the activation of inflammatory pathways. In particular, the increased production of proinflammatory cytokines, including TNF- α and IL-1 β , may contribute to the development and progression of VPA-induced hepatic and renal damage, highlighting the potential role of inflammation in VPA-mediated organ toxicity. In our study, VPA administration significantly increased TNF- α and IL-1 β levels in both liver and kidney tissues. Treatment with MYR50 and MYR100 significantly attenuated these VPA-induced increases in pro-inflammatory cytokine levels, indicating that myricetin may exert a protective effect against VPA-induced inflammatory responses in both tissues.

JNK is activated in response to various cellular and environmental stimuli, including proinflammatory cytokines, oxidative stress, changes in osmolarity or cellular metabolism,

DNA damage, ultraviolet radiation, ischemia, and heat shock. In particular, JNK is strongly induced by proinflammatory cytokines, including IL-1 β and TNF- α , as well as cellular stressors such as reactive oxygen species, and is therefore referred to as a stress-activated protein kinase (Wada & Penninger, 2004; Khaleel et al., 2023). p38 MAPK is activated in response to various cellular and environmental stressors, including changes in osmolarity or metabolism, DNA damage, heat shock, ischemia, proinflammatory cytokines, ultraviolet irradiation, and oxidative stress. Activation of p38 MAPK generally leads to inflammatory responses or apoptosis; however, it also regulates various other cellular processes, including cell proliferation, differentiation, adhesion, and migration (Wong & Cheng, 2005; Chen et al., 2025).

Our findings indicate that JNK exhibited a distinct response pattern compared with p38 MAPK. While renal JNK levels did not differ significantly among the experimental groups, hepatic JNK levels showed an increasing trend following VPA administration; however, this change did not reach statistical significance. Furthermore, MYR treatment, particularly at the 100 mg/kg dose, was associated with a further tendency toward increased hepatic JNK levels. These findings suggest that the potential protective effect of MYR against VPA-induced tissue injury may not be mediated through the suppression of JNK signaling. Although JNK is an important signaling molecule involved in the regulation of cellular stress responses, inflammation, and apoptosis, its activation may vary depending on tissue type and cellular conditions. Therefore, the differential JNK response observed in our study may reflect tissue-specific characteristics of VPA-induced injury. In contrast, the marked increase in p38 MAPK levels following VPA administration and the significant attenuation of this increase by MYR treatment in both hepatic and renal tissues suggest that the p38 MAPK pathway may play a more prominent role than JNK in the protective effects of MYR against VPA-induced tissue injury.

Apoptosis, commonly referred to as programmed cell death, is an essential biological process that plays a critical role in embryonic development as well as in maintaining the homeostasis, survival, and functional integrity of mature tissues. Reactive oxygen species (ROS), which are predominantly generated within mitochondria, can significantly contribute to the initiation and progression of apoptotic processes. The mitochondrial pathway of apoptosis, one of the major mechanisms regulating programmed cell death, is tightly controlled by members of the Bcl-2 protein family (Akaras et al., 2023; Simsek & Akaras, 2023). The regulation of apoptosis involves not only pro-apoptotic molecules but also anti-apoptotic

proteins. Among the key anti-apoptotic regulators, Bcl-2 contributes to the stabilization of the mitochondrial outer membrane, thereby preventing cytochrome c release and protecting cells against apoptosis (O'Brien & Kirby, 2008).

In a study conducted by Azirak et al., (2025) a reduction in Bcl-2 levels was observed in VPA-induced hepatic injury. Similarly, Dai et al. (2017) reported that VPA exposure decreased Bcl-2 expression at both the mRNA and protein levels. Comparable findings have also been reported in the literature. In contrast, our findings differ from these previous reports, as VPA administration resulted in increased Bcl-2 levels in both hepatic and renal tissues in our study. In our study, we observed that VPA administration increased Bcl-2 levels in both hepatic and renal tissues. While VPA administration significantly increased renal Bcl-2 levels, treatment with MYR50 and MYR100 significantly attenuated this increase, bringing Bcl-2 levels closer to those observed in the control group. In hepatic tissue, although MYR treatment tended to reduce Bcl-2 levels, these reductions were not statistically significant compared with the VPA group. An important point should be considered when interpreting our Bcl-2 findings. Bcl-2 is an anti-apoptotic protein; therefore, the observed increase in Bcl-2 levels following VPA administration may reflect an adaptive anti-apoptotic response to VPA-induced cellular stress. In this context, the attenuation of Bcl-2 levels following MYR treatment, particularly in renal tissue, may indicate normalization of the cellular response to VPA-induced stress rather than a direct pro-apoptotic effect of MYR.

Lipid peroxidation occurs through the oxidation of polyunsaturated fatty acids by reactive oxygen species (ROS), resulting in the formation of toxic end products such as MDA. An increase in MDA levels is widely recognized as a biomarker of oxidative damage to cellular structures. Previous studies have reported that VPA induces hepatic injury (Ahangar et al., 2017), causes intestinal toxicity (Oktay et al., 2015), and induces neurotoxicity (Tunali et al., 2020). A common feature of these toxic effects is an increase in MDA levels, suggesting that oxidative stress and lipid peroxidation may play an important role in VPA-induced tissue injury. Several studies have investigated the effects of myricetin on lipid peroxidation. Pandey et al. (2009) reported that myricetin treatment reduced MDA levels in erythrocytes, indicating its potential to attenuate oxidative damage. Similarly, Alfwuaires and Algefare (2026) investigated the effects of MYR in an experimental model of hepatic injury and observed a significant reduction in hepatic MDA levels following MYR treatment. Our findings regarding MDA

levels are consistent with these previous reports, further supporting the potential of myricetin to attenuate lipid peroxidation and oxidative stress.

In this study, VPA exposure significantly increased MDA levels in rat liver and kidney tissues, indicating enhanced lipid peroxidation. Treatment with MYR at doses of 50 and 100 mg/kg significantly reduced MDA levels in both hepatic and renal tissues. When the Bcl-2 and MDA findings obtained in our study are considered together, the increase in Bcl-2 levels observed following VPA exposure may reflect a compensatory cellular defense response against oxidative and mitochondrial stress. Given that Bcl-2 is an anti-apoptotic protein that plays an important role in maintaining mitochondrial integrity, the concomitant increases in MDA and Bcl-2 levels suggest that VPA-induced oxidative stress may have activated endogenous anti-apoptotic defense mechanisms in the cells. The protective effects of both MYR doses may primarily be associated with the attenuation of VPA-induced oxidative stress and lipid peroxidation, while the reduction in oxidative stress may have diminished the cellular need for a compensatory anti-apoptotic Bcl-2 response.

In our study, histopathological evaluations, consistent with the biochemical findings, demonstrated the protective effects of MYR against VPA-induced liver and kidney injury. Histopathological examination revealed that VPA administration caused marked structural damage in both liver and kidney tissues, characterized by degeneration, inflammation, and necrosis. MYR treatment attenuated the histopathological alterations in both tissues, with MYR100 exhibiting a more pronounced protective effect. These findings suggest that the protective effects of MYR may be associated with its ability to attenuate VPA-induced cellular injury and inflammatory responses in both tissues. The greater efficacy observed with MYR100 further supports a potential dose-dependent protective effect of MYR against VPA-induced hepatic and renal damage.

MYR may have the potential to inhibit PERK kinase activity by forming stable hydrogen bonds with critical regions within the kinase pocket, such as the P-loop (residues 601–608) and the VAIK motif (Lys622). However, the high affinity exhibited by synthetic inhibitors, particularly AMG44 and GSK-series compounds, is likely attributable to their specialized chemical structures, which enable them to extend into the deeper back pocket of the enzyme. Since myricetin lacks the molecular length and hydrophobic extensions required to establish such deep-pocket interactions, its binding affinity remains relatively limited compared with that

of synthetic counterparts. Nevertheless, its more stable binding relative to ATP suggests that MYR may have potential as a naturally derived PERK inhibitor with a potentially favorable toxicity profile. These findings support the potential of MYR as an ATP-competitive inhibitor of PERK (EIF2AK3). The calculated binding energy of -7.52 kcal/mol indicates that MYR has a favorable binding propensity that may enable it to compete with the natural substrate ATP for the enzyme's binding pocket. Although MYR may not provide the same degree of deep-pocket stabilization as synthetic drug candidates such as AMG44, its observed affinity highlights the potential importance of natural compounds in the modulation of ER stress-related pathways.

Furthermore, myricetin is predicted to be a potential inhibitor of the IRE1 α kinase domain, exhibiting stable binding within the kinase domain while restricting the flexibility of the binding pocket without substantially disrupting the active conformation of the protein. By reducing the distances between critical catalytic residues, MYR may influence the enzymatic activity of IRE1 α . The 500-ns molecular dynamics simulation further demonstrated that MYR exhibited favorable structural and dynamic properties as a potential IRE1 α inhibitor, supporting its potential role in modulating IRE1 α -mediated signaling.

Conclusion

When the biochemical, histopathological, and molecular docking findings of our study are considered together, VPA exposure appears to induce marked hepatic and renal injury associated with oxidative stress, inflammation, and cellular stress responses. These findings suggest that MYR may have strong protective potential against VPA-induced tissue injury, particularly through the suppression of ER stress and inflammatory responses. In addition, molecular docking and molecular dynamics analyses demonstrated that MYR can establish stable interactions with key ER stress sensors, such as PERK and IRE1, supporting its potential to modulate the activity of these proteins. In conclusion, MYR may be considered a naturally derived candidate compound with potential efficacy in preventing or attenuating VPA-induced hepatic and renal toxicity by simultaneously targeting oxidative stress, inflammation, and ER stress. However, further molecular and experimental studies are required to confirm the direct inhibitory effects of MYR on PERK and IRE1 and to elucidate its therapeutic potential.

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Conflict of Interest

The authors declared no conflicts of interest.

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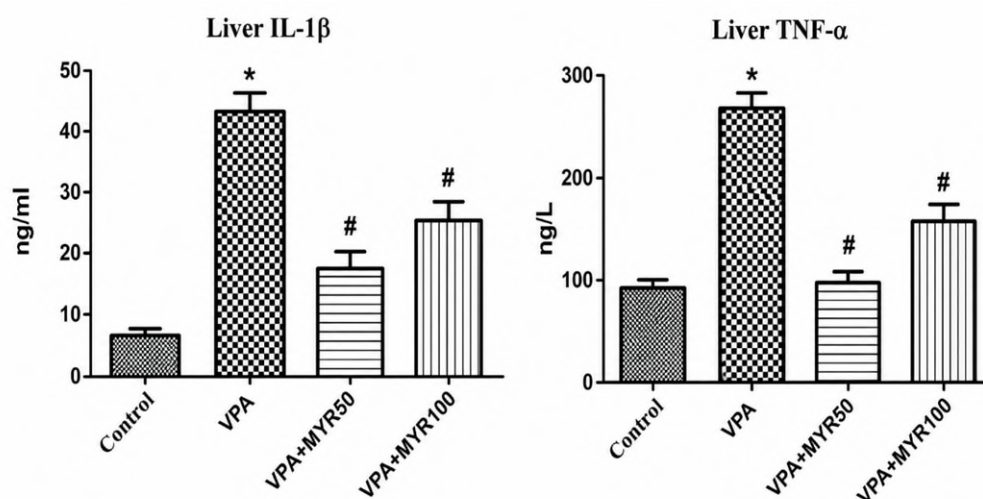


Fig. 1 Effects of MRY treatment on hepatic IL-1 β and TNF- α levels in VPA-induced rat

Data are presented as mean $\pm$ SD. * $p < 0.05$ versus the control group; # $p < 0.05$ versus the VPA group. IL-1 β , interleukin-1 beta; TNF- α , tumor necrosis factor-alpha; VPA, valproic acid; MYR50 and MYR100, 50 and 100 mg/kg myricetin, respectively.

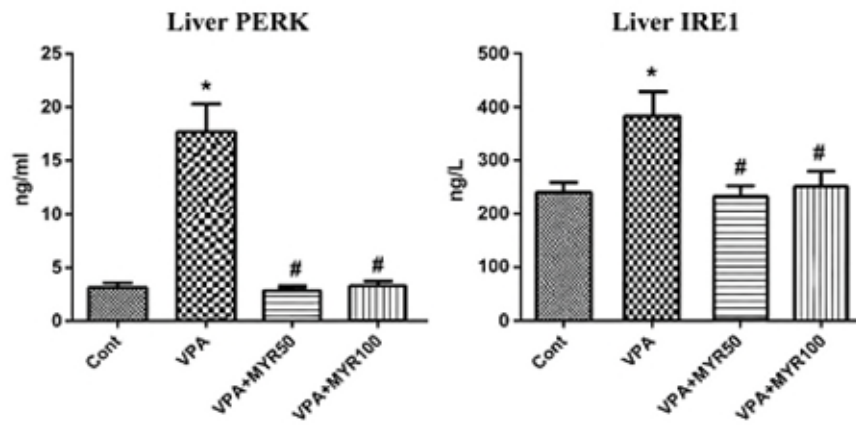


Fig. 2 Effects of MRY treatment on hepatic PERK and IRE1 levels in VPA-induced rat

Data are presented as mean $\pm$ SD. * $p < 0.05$ versus the control group; # $p < 0.05$ versus the VPA group. PERK, protein kinase R-like endoplasmic reticulum kinase; IRE1, inositol-requiring enzyme; VPA, valproic acid; MRY50 and MRY100, 50 and 100 mg/kg myricetin, respectively.

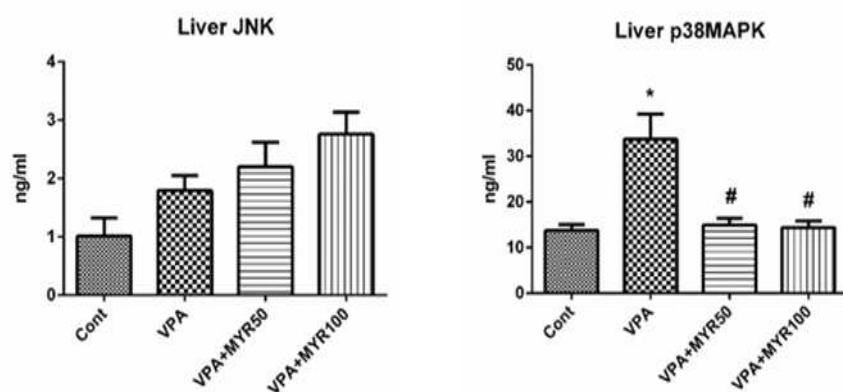


Fig. 3 Effects of MRY treatment on hepatic JNK and p38MAPK levels in VPA-induced rat

Data are presented as mean $\pm$ SD. * $p < 0.05$ versus the control group; # $p < 0.05$ versus the VPA group. JNK, c-Jun N-terminal kinases; p38MAPK, p38 mitogen-activated protein kinase; VPA, valproic acid; MYR50 and MYR100, 50 and 100 mg/kg myricetin, respectively.

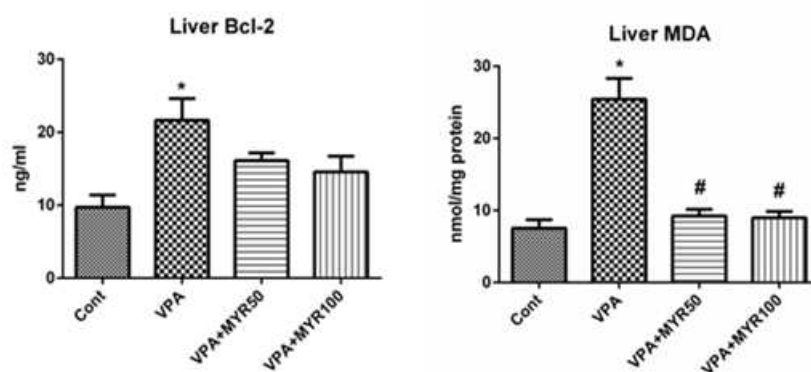


Fig. 4 Effects of MRY treatment on hepatic Bcl-2 and MDA levels in VPA-induced rat

Data are presented as mean $\pm$ SD. * $p < 0.05$ versus the control group; # $p < 0.05$ versus the VPA group. Bcl-2, b-cell lymphoma 2; MDA, malondialdehyde; VPA, valproic acid; MYR50 and MYR100, 50 and 100 mg/kg myricetin, respectively.

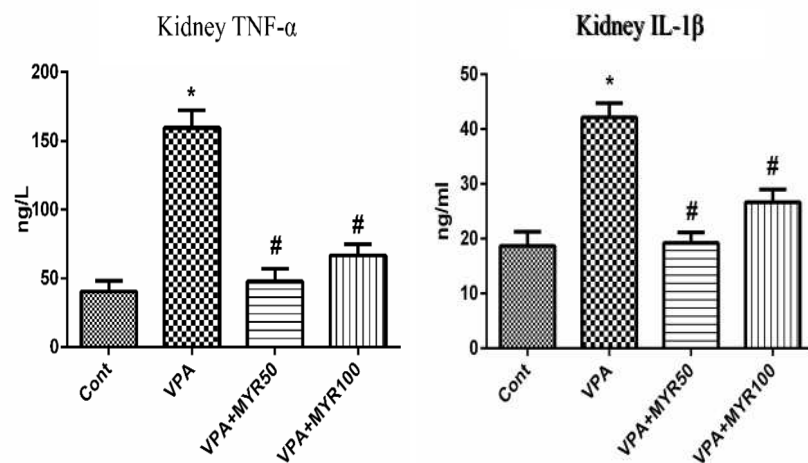


Fig. 5 Effects of MRY treatment on renal IL-1 β and TNF- α levels in VPA-induced rat

Data are presented as mean $\pm$ SD. * $p < 0.05$ versus the control group; # $p < 0.05$ versus the VPA group. IL-1 β , interleukin-1 beta; TNF- α , tumor necrosis factor-alpha; VPA, valproic acid; MRY50 and MRY100, 50 and 100 mg/kg myricetin, respectively.

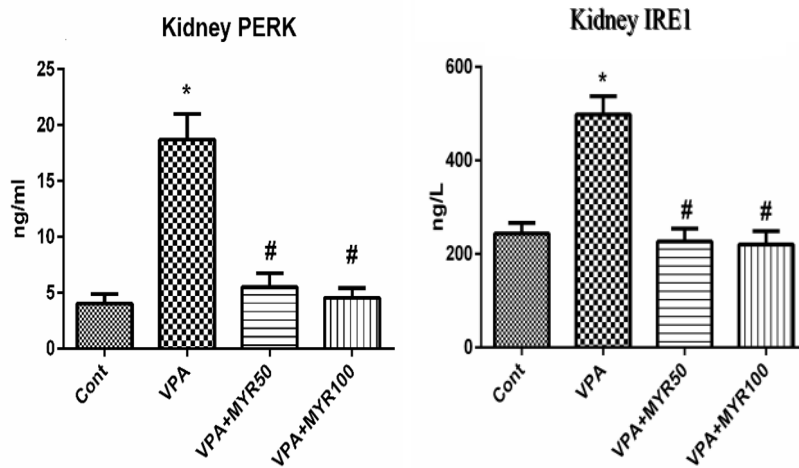


Fig. 6 Effects of MRY treatment on renal PERK and IRE1 levels in VPA-induced rat

Data are presented as mean $\pm$ SD. * $p < 0.05$ versus the control group; # $p < 0.05$ versus the VPA group. PERK, protein kinase R-like endoplasmic reticulum kinase; IRE1, inositol-requiring enzyme; VPA, valproic acid; MYR50 and MYR100, 50 and 100 mg/kg myricetin, respectively.

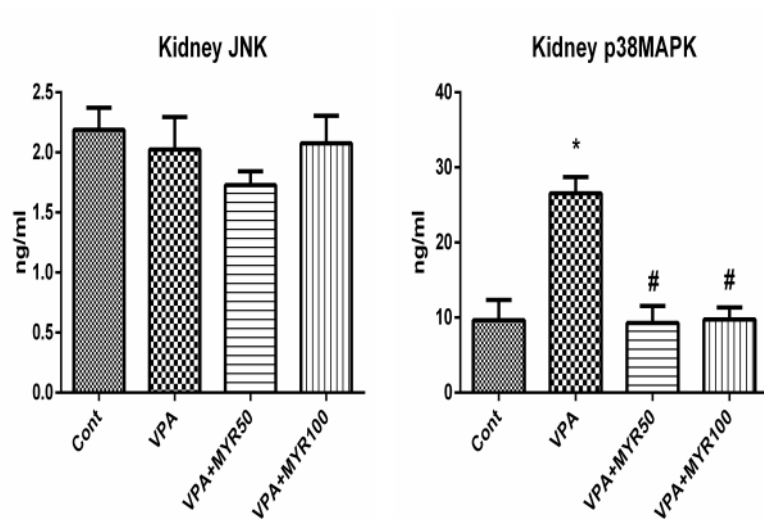


Fig. 7 Effects of MRY treatment on renal JNK and p38MAPK levels in VPA-induced rat

Data are presented as mean $\pm$ SD. * $p < 0.05$ versus the control group; # $p < 0.05$ versus the VPA group. JNK, c-Jun N-terminal kinase; p38 MAPK, p38 mitogen-activated protein kinase; VPA, valproic acid; MYR50 and MYR100, 50 and 100 mg/kg myricetin, respectively.

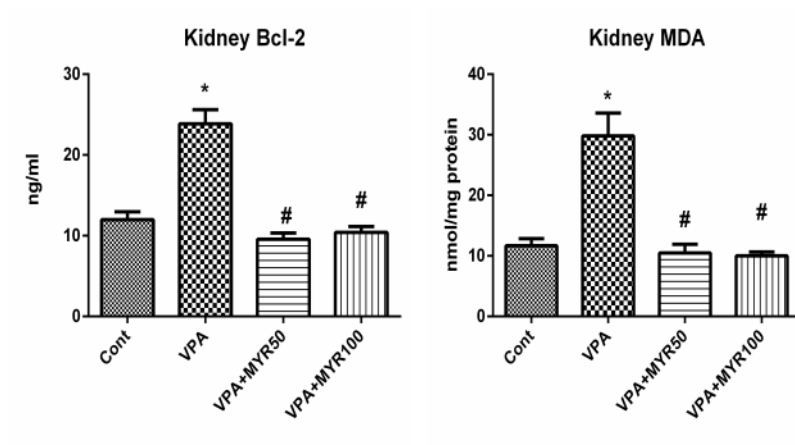


Fig. 8 Effects of MRY treatment on renal Bcl-2 and MDA levels in VPA-induced rat

Data are presented as mean $\pm$ SD. * $p < 0.05$ versus the control group; # $p < 0.05$ versus the VPA group. Bcl-2, b-cell lymphoma 2; MDA, malondialdehyde; VPA, valproic acid; MRY50 and MRY100, 50 and 100 mg/kg myricetin, respectively.

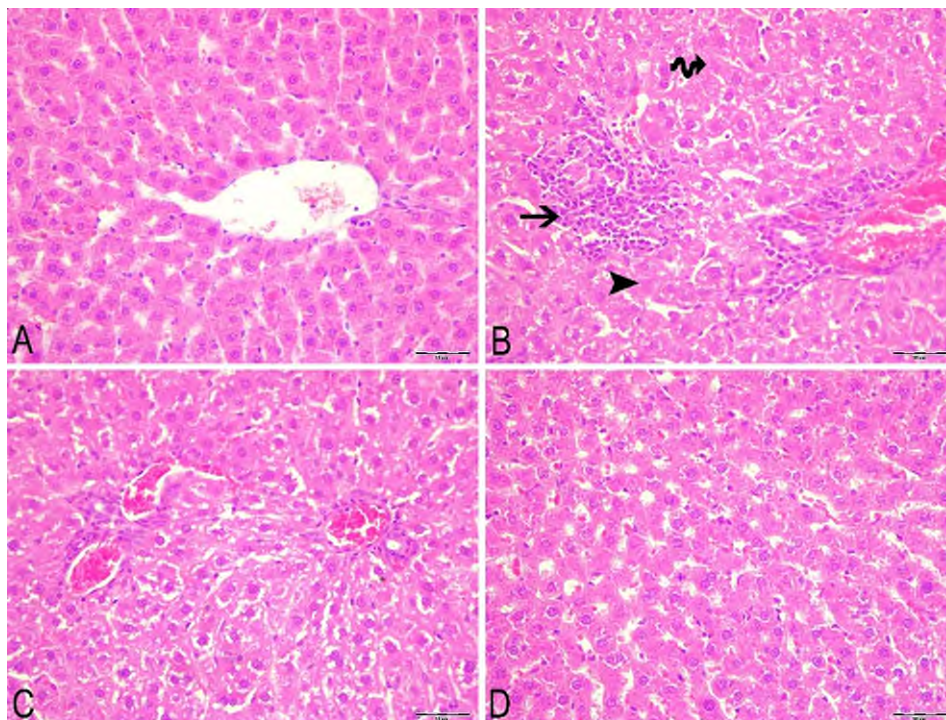


Fig. 9 Histopathological examination of liver tissues in the experimental groups.

Liver tissue, control (Fig A), VPA (Fig B), VPA+MYR50 (Fig C) and VPA +MYR100 (Fig D), Inflammation (arrow), necrosis (curved arrow), and degeneration (arrowhead). H&E, bar: 40µm.

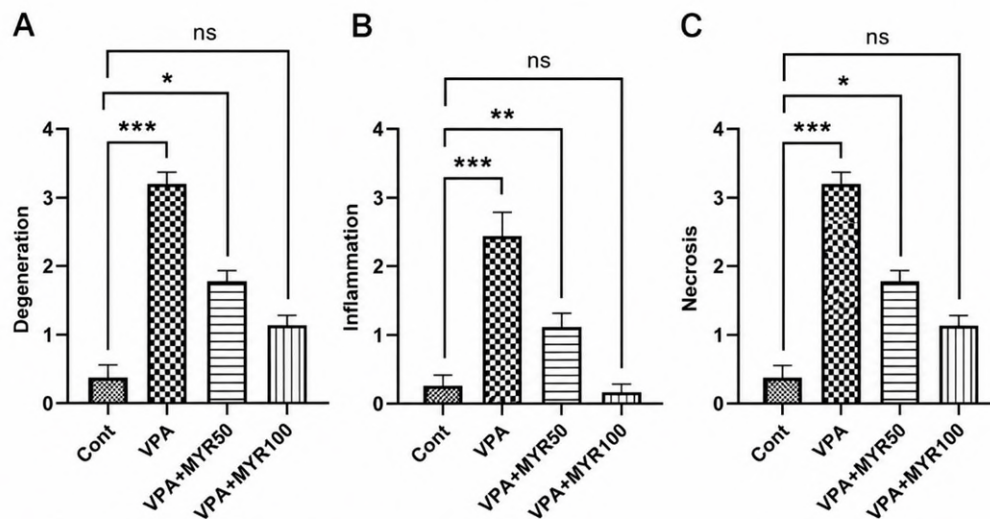


Fig. 10 Histopathological scores of degeneration, inflammation, and necrosis in liver tissues of the experimental groups.

Semi-quantitative histopathological scores of degeneration (A), inflammation (B), and necrosis (C) in liver tissues of the Control (Cont), VPA, VPA+MYR50, and VPA+MYR100 groups. Data are presented as mean $\pm$ SD. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant.

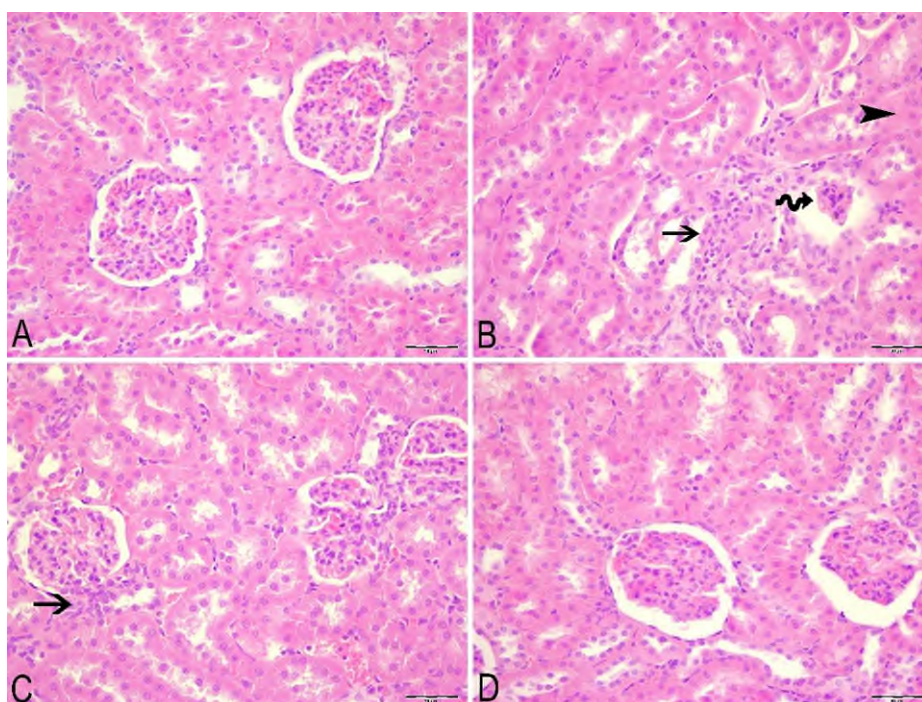


Fig. 11 Histopathological examination of kidney tissues in the experimental groups.

Kidney tissue, control (Fig A), VPA (Fig B), VPA+MYR50 (Fig C) and VPA +MYR100 (Fig D), Inflammation (arrow), glomerular atrophy (curved arrow), and tubular degeneration (arrowhead). H&E, bar: 40µm.

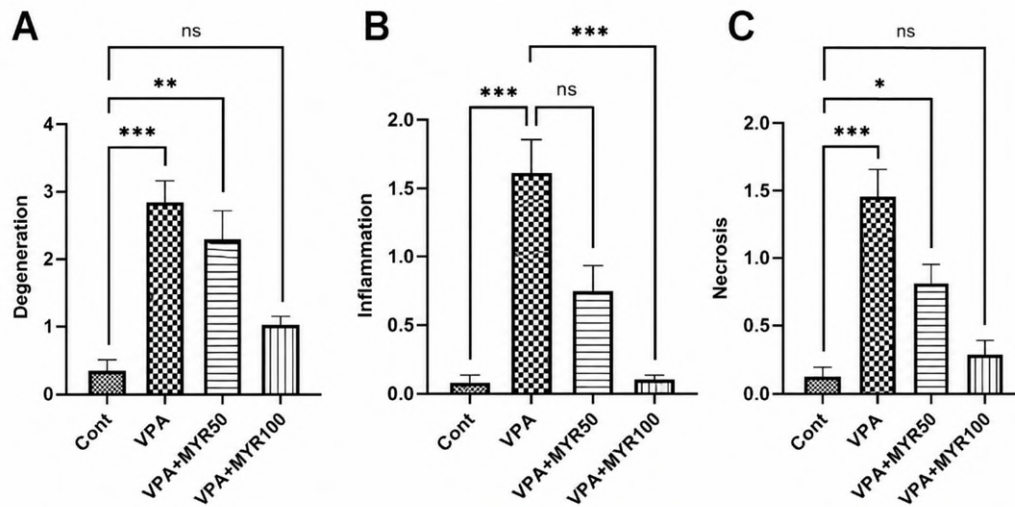


Fig. 12 Histopathological evaluation of renal degeneration, inflammation, and necrosis in the experimental groups.

Semi-quantitative histopathological scores of renal degeneration (A), inflammation (B), and necrosis (C) in the Control (Cont), VPA, VPA+MYR50, and VPA+MYR100 groups. Data are presented as mean $\pm$ SD. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ns, not significant.

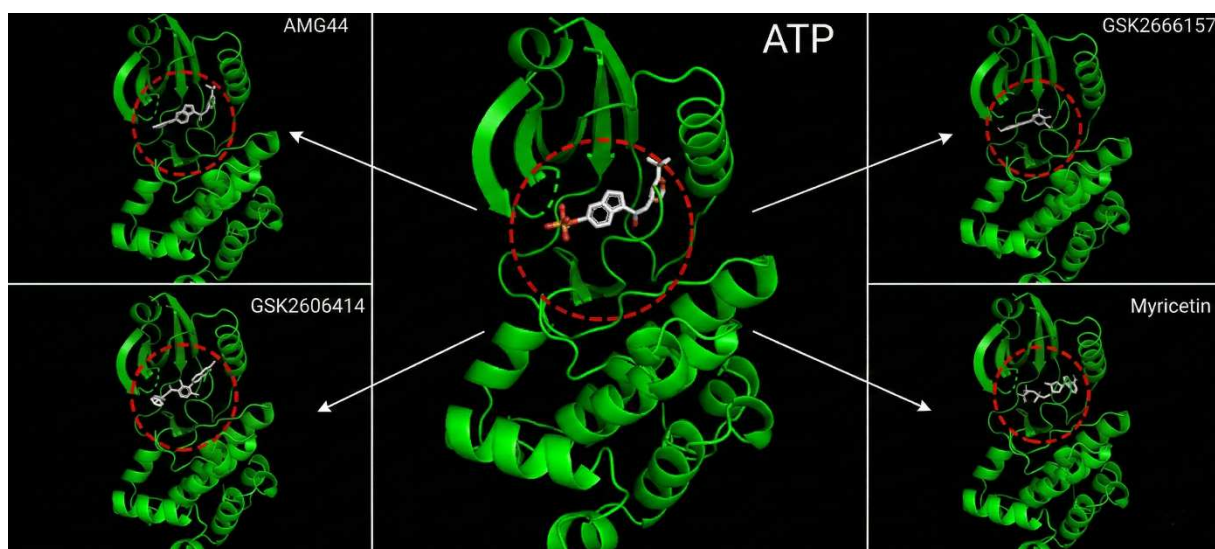


Fig. 13 Comparison of ATP and inhibitor binding in PERK active site.

The central panel shows ATP-bound protein kinase, with inhibitors (AMG44, GSK2606414, GSK2666157, and Myricetin) shown in surrounding panels. Red dashed circles indicate the binding pockets.

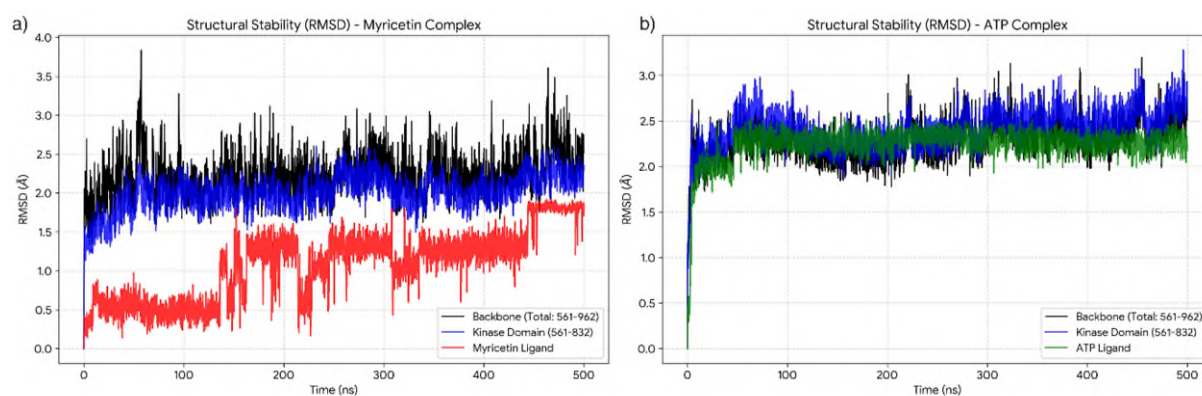


Fig. 14 Root mean square deviation (RMSD) profiles of the (A) IRE1 α –ATP and (B) IRE1 α –myricetin complexes during the 500 ns molecular dynamics (MD) simulations. The RMSD trajectories illustrate the time-dependent structural stability of the entire protein backbone (residues 561–962), the kinase domain (residues 561–832), and the bound ligand throughout the simulation period.

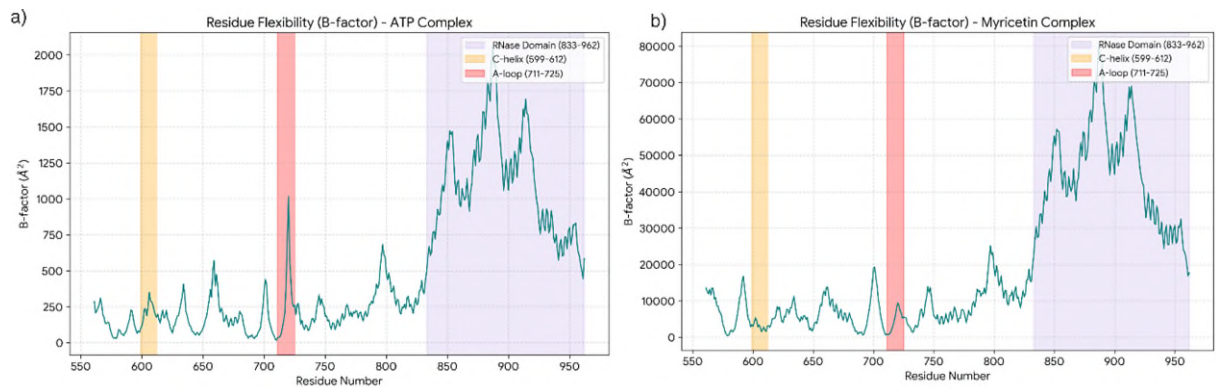


Fig. 15 a) IRE1 α -ATP b) Residue-wise B-factor distribution of the cytoplasmic region (residues 561–962) of the IRE1 α -myricetin complex. The RNase domain (residues 833–962, purple), α C-helix (residues 599–612, orange), and activation loop (A-loop; residues 711–725, red) are highlighted.

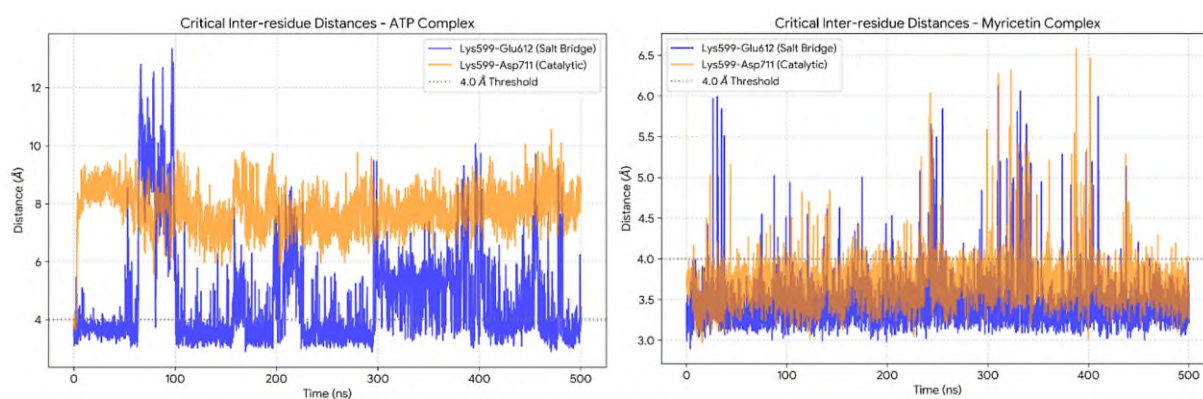


Fig. 16 Time-dependent distance profiles of critical active-site residues during the 500 ns molecular dynamics simulation. The Lys599–Glu612 salt bridge (blue), which characterizes the active (DICI) conformation of the kinase domain, and the Lys599–Asp711 catalytic interaction (orange) are shown.

Supplementary Files

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